

Accomplishments: 1994-present

1994: Receptor Assays for Domoic Acid and PSP Toxins

New receptor-based assays for domoic acid and PSP toxins have been found to be useful for detecting toxins in toxic algae, shellfish, crab hepatopancreas, and the serum of exposed humans and animals. These high capacity assays are formatted to contain 96 data points on a 3 x 4" filter card to provide rapid, reliable results and detect all toxin congeners in a manner quantitatively proportional to their toxicity. These assays are anticipated to be used in dock side testing and confirmation of marine toxin exposure in humans and marine animals.

Contact: Fran Van Dolah

Brain Mapping Studies of Adverse Effects of Ciguatoxins and Domoic Acid

The immediate response gene c-fos has been utilized to map the neuronal pathways activated by marine toxins in laboratory animals. Using this approach, two major brain regions have been identified to be the targets of amnesic shellfish poisoning; the hippocampus which controls memory processing and the nucleus solitarius which regulates gastrointestinal function. The hippocampus was determined to be irreversibly damaged by this toxin whereas the nucleus solitarius is not. Analogous studies with ciguatoxin have indicated that this toxin activates medial preoptic region of the brain controlling thermoregulation. These studies are being used to better assess the risk of marine toxins to seafood consumers.

Contact: John Ramsdell

1995: Detection of Domoic Acid and PSP Toxin Activity in Algae and Animals

New rapid and inexpensive receptor-based assays for domoic acid and PSP toxins have been found to be reliable for detecting toxins in toxic algae, shellfish, crab hepatopancreas, and the serum and urine of exposed humans and animals. These assays have been validated against HPLC analytical methods and mouse bioassay. National reference laboratories within the European Community have requested that NMFS offer training workshops on implementing the receptor assays and expressed a desire to initiate collaborative testing programs. These assays are anticipated to be used in dockside testing of shellfish and confirmation of marine toxin exposure in seafood consumers.

Contact: Fran Van Dolah

Neonates are at High Risk to Domoic Acid Effects

The hazards of marine toxin exposure to seafood consumers have been further elucidated through the use of biomarkers. Two major brain regions have been identified to be the targets of amnesic shellfish poisoning; one controlling memory processing and the other regulating gastrointestinal function. Computer image analysis and reconstruction of this data base has generated three dimensional visualizations of brain damage caused by marine toxin exposure. Neonatal animals have been determined to be ten times more susceptible to amnesic shellfish poisoning. Amnesic shellfish poison (domoic acid) has been determined to be cleared from the serum within hours, and multiple exposures have been found not to change the clearance rate. Domoic acid given to lactating rats passes from the blood to the urine, but is found in very low concentrations in the milk. This indicates that although neonates are highly susceptible to the toxin effects of domoic acid, breast milk may not be a likely route of exposure. These studies are being used to better assess the risk of marine toxins to seafood consumers.

Contact: John Ramsdell

1996: Cloned Glutamate Receptor Used for Domoic Acid Receptor Assay

Receptor-based assays for marine biotoxins have been further optimized this year. The assay for domoic acid has been modified to utilize a cloned glutamate receptor, and addition of glutamate decarboxylase to sample extracts has been demonstrated to be useful in removing potential interference due to glutamate. The assay has been utilized successfully by the NMFS Seattle Laboratory for field studies on domoic acid production in diatoms. Receptor assays were demonstrated at the NATO Advanced Study Institute on Harmful Algal Blooms, held in Bermuda, May 28-June 6. Feasibility of using receptor assays for shipboard monitoring of algal blooms was demonstrated on a research cruise aboard the R/V Pelican in the Gulf of Mexico, Sept 6-15.

Contact: Fran Van Dolah

Hazard of Repeated Domoic Acid Exposures

The hazards of marine toxin exposure to specific groups of seafood consumers has been further elucidated, this time investigating the potential hazard to consumers who may receive repeated subsymptomatic exposures to amnesic shellfish poisoning (domoic acid). A battery of tests including toxicokinetics, scored symptomatology, working memory assessment and neurodegeneration analysis and neurodegeneration analysis were used as endpoints to evaluate enhanced toxicity to four repeated exposures to domoic acid. Experiments included both four subsymptomatic and four symptomatic doses and were conducted in two strains of mice, one of which is highly susceptible to drug induced seizures. Repeated exposures did not produced enhanced toxicity in any of the four endpoints, indicating that each exposure is an independent event that is nonadditive. These results were presented in September to Canadian and U.S. regulatory officials.

Contact: John Ramsdell

1997: Collaborative Testing of Receptor Assays for Marine Toxins

Receptor based assays for PSP, ASP, NSP, and CFP have been developed and laboratory validation completed in the past four years. These assays are now ready to be tested corroboratively in formal interlaboratory trials. The first of these trials, testing the assay for NSP in oysters, has been initiated as an AOAC Peer Verified Method trial, which will be completed in FY1998.

Contact: Fran Van Dolah

Domoic Acid Producing Pseudo-nitzschia Identified in the Gulf of Mexico

Pseudo-nitzschia spp. were found to be abundant in Louisiana coastal samples. At shelf sites, these species were present in 70% of all samples and concentrations were minimal in spring, often exceeding one million cells/liter. Among those species identified were the toxic *P. multiseries*, *P. delicatissima*, and *P. pseudodelicatissima*. The DA receptor assay (corroborated by HPLC analyses) detected DA in phytoplankton samples containing *Pseudo-nitzschia* spp. from both estuarine and shelf sites. Future work is aimed at addressing the issues of how environmental factors influence DA production by *Pseudo-nitzschia* spp. in natural populations, and why no outbreaks of DA poisoning have occurred in this region. This project is in collaboration with Dr. Q. Dortch (LUMCON).

Contact: Greg Doucette

1998: Identification of Domoic Acid in California Sea Lions and their Foodweb

Through the combined use of rRNA probes for *Pseudo-nitzschia* spp., a receptor binding assay and a newly developed liquid chromatography-mass spectrometry method to definitively identify domoic acid, a toxic bloom of *Pseudo-nitzschia australis* occurring this past spring in Monterey Bay, CA was tracked throughout the event. Concurrent use of these novel detection methods, a collaborative effort with the Monterey Bay Aquarium Research Institute, comprises a powerful approach to acquiring near real time data for harmful algal blooms. A time course for the bloom and associated toxin levels was developed, which showed temporal changes in bloom toxicity and the subsequent appearance of a non-toxic species, *Pseudo-nitzschia pseudodelicatissima*, along with a concomitant decline in DA levels. In addition, the *P. australis* bloom was demonstrated to be the source of domoic acid transmitted through planktivorous fish (e.g., anchovies) and ultimately to sea lions in the region, which suffered a mortality event in excess of 50 animals. The latter was the first documentation of naturally incurred DA in mammalian body fluids, providing compelling evidence that the cause of sea lion mortalities was due to DA poisoning.

Contact: Greg Doucette

1999: Tandem Mass Spectrometric Identification of Domoic Acid: Absolute Identification of Domoic Acid in California Sea Lions

A newly developed liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was used to provide positive identification of domoic acid in living marine resources and protected species following a toxic bloom of *Pseudo-nitzschia australis* that occurred in spring 1998 in Monterey Bay, CA. The toxic bloom was associated with a sea lion mortality event that resulted in the loss of over 50 animals. The LC-MS/MS method is based upon the detection of highly specific fragmentation products from the collisionally induced dissociation of domoic acid pseudo-molecular ions. The LC-MS/MS method allowed sensitive detection of domoic acid in a variety of matrices ranging from sea lion tissue and excrement to other components of the food web, including planktivorous fish (e.g., anchovies). This is the first utilization of LC-MS for domoic acid identification and is substantial advance in detection methodology because it provides the absolute identification of toxin that is not possible with functional assays or other HPLC-coupled detection methods.

Contact: Peter Moeller

Isolation of a Domoic Acid Producing Diatom From Louisiana Shelf Waters

Screening of eighteen clonal *Pseudo-nitzschia* cultures established by colleagues at LUMCON revealed production of DA by two of these clones, both identified as *P. pseudodelicatissima* - a species only twice reported as toxic and never verified by mass spectrometry. Both cultures, isolated from LA shelf waters, were confirmed by tandem mass spectrometry to produce DA. Moreover, the patterns of DA production in culture showed the highest cellular toxin levels during exponential growth and the lowest during stationary phase B essentially the opposite of findings reported previously for most other toxic *Pseudo-nitzschia* spp. This pattern is likely due to toxin being excreted into the growth medium during stationary phase; however, since toxin levels are very low, we are currently attempting to reduce our limit of DA detection in seawater to permit the measurement of toxin in the medium. These observations of DA production by exponentially growing cultures have important implications for the toxicity of rapidly growing field populations dominated by this species.

Contact: Greg Doucette

2000: Determination of Domoic Acid in Mole Crabs (*Emerita analoga*): A Possible New Indicator Species

We are currently examining the trophic transfer of domoic acid (DA) through the pelagic and benthic food webs in Monterey Bay, California, a site of recurrent *Pseudo-nitzschia* blooms and marine mammal mortalities associated with these HAB events. One aspect of this work, being done in collaboration with CA State Univ. at Monterey Bay and the Univ. of CA at Santa Cruz, is to evaluate use of the mole crab (*Emerita analoga*), as an indicator species for DA, since the current sentinel organism, the intertidal blue mussel (*Mytilus californianus*), shows minimal or undetectable toxicity during local bloom events. We have developed a new extraction protocol for the determination of DA in mole crabs that yields toxin recoveries of 96.5 ± 2.9 percent. We have also confirmed by LC-MS/MS that mole crabs accumulated measurable amounts of DA during toxic *Pseudo-nitzschia* bloom events (0.5-10 : g DA/g tissue), while the blue mussel showed no detectable toxin. In addition, the rise and fall of DA toxicity in mole crabs coincided with similar changes in *Pseudo-nitzschia* cell concentrations. Extensive field trials are now underway to further compare the DA toxicity of mole crabs vs. blue mussels and its correlation to the presence of toxic *Pseudo-nitzschia* cells, and to critically evaluate the potential use of mole crabs as the sentinel species for coastal DA events in this area. These data will also be useful in establishing the effectiveness of mole crabs as a vector for transferring DA to higher trophic levels.

Contact: Greg Doucette

Identification Of Domoic Acid In Grey Whales And Sea Lions In California Coastal Waters

Using DA receptor assays as a rapid screen and HPLC-MS/MS for chemical confirmation, we were able to confirm that stranded gray whales and sea lions were exposed to DA at concentrations that might be expected to cause adverse effects. We previously demonstrated that domoic acid was the causative agent of a California sea lion mortality event in 1998. This year for the first time, DA poisoning appears to have had widespread impacts on several different marine mammal species with diverse feeding habits and geographic distributions: gray whales stranded in San Francisco Bay in April-May, sea otters that died in the same area during the same time frame, sea lions stranded in San Luis Obispo County in June-early July, and sea lions stranded 100 miles farther south in Ventura/Los Angeles Counties in late July. Most of the sea lions were in good body condition, but displayed seizure and scratching activities documented previously in the 1998 sea lion mortality event. Blooms of the DA producing diatom *Pseudonitzschia australis* were found in California coastal waters concurrently with the marine mammal morbidities/mortalities.

Contact: Tod Leighfield

2002: Identification of Domoic Acid as a Causative Agent in Mass Mortalities of Marine Mammals on the California Coast

Extensive mortalities of marine mammals along the California coast occurred this year from February through June 2002. The event began with the mysterious deaths of dolphins along the California coast from San Luis Obispo to Orange County. Analysis of dolphin urine by the Marine Biotoxins Program's Analytical Response Team (ART) revealed high concentrations of the algal toxin, domoic acid (DA). Blooms of the DA-producing diatom, *Pseudo-nitzschia australis*, were subsequently found to be the source of toxicity and these blooms continued intermittently along the California coast through June, resulting in the deaths of large numbers of dolphins, sea lions, sea otters, and whales. Over 100 samples were analyzed by the ART to confirm the involvement of DA in mortalities of novel marine species and provide insight into trophic transfer of DA to dolphins and placental transfer of the toxin in gestating female sea lions. DA was previously identified by this Program as the causative agent in the mass mortality of California sea lions in the Monterey Bay area in 1998 and of California sea lions and sea otters in 2000. This is the first year in which multiple species of marine mammal were severely impacted from San Francisco south to Los Angeles along the central California coast, as well as the Baja, California coast and into Mexico.

Contact: Fran Van Dolah

Transfer of Receptor Assay Technology to SW African Countries Initiated

The southwest African countries of South Africa, Namibia, and Angola have either historical or recently emerging problems with one or more groups of marine algal toxins. These countries have requested assistance through the U.N. International Atomic Energy Agency (IAEA) in establishing capabilities for receptor assay-based detection of algal toxins in seafood products. A project planning meeting was held at IAEA Headquarters in Vienna, Austria to develop a regional technical cooperation proposal for the transfer of the Marine Biotoxins Program's receptor assay technology to each of these three African countries. This project will be modeled after an ongoing IAEA-sponsored program in SE Asia, with the African end-users visiting the CCEHBR laboratory next year for training and returning to their home institutions to begin conducting the assays. An inter-calibration study coordinated through our Program will follow, and then receptor assays will be implemented as a component of their respective toxin monitoring programs, which are either well established (S. Africa) or currently being developed. Acquisition of receptor-based technology will be of immediate benefit to each of our African partners, given their rapidly growing fishery and aquaculture industries along with the accompanying demands for biotoxin testing of products for export to world markets.

Contact: Fran Van Dolah

Volunteer Monitor Harmful Phytoplankton Along South Carolina Coast

The South Carolina Phytoplankton Monitoring Network (SCPMN) began its second year of existence with over 34 groups monitoring state coastal waters for potentially harmful algal species. A total of over 50 sampling sites from all coastal counties of South Carolina are monitored each week. Volunteer groups are composed of both middle and high school students, state park personnel, and citizen environmental groups. This NOAA sponsored community program serves to increase the awareness of constituent groups about the many issues related to harmful algae and directly involves volunteers in coastal stewardship. In the SCPMN's first year of existence, volunteers observed three potentially toxic algae, including *Pseudo-nitzschia*, *Dinophysis*, and *Prorocentrum lima*. Observation and identification of phytoplankton along the South Carolina coast will be useful in developing a species list and record of distribution, as well as alerting NOAA scientists to the presence of potentially harmful species at the many sampling sites.

Contact: Steve Morton

2003: Domoic Acid-Induced Gene Expression in Mouse Brain Identified by DNA Microarray Technology

Domoic acid is responsible for amnesic shellfish poisoning in humans and is the causative agent of extensive marine mammal mortalities. Domoic acid is an excitatory neurotoxin that mimics the neurotransmitter glutamate and is a potent activator of certain subtypes of brain glutamate receptors. Persistent activation of these receptor subtypes results in calcium dependent cell death and neuronal lesions in areas of the brain where these receptors are heavily concentrated. To better understand the mechanisms involved in the response to toxic levels of domoic acid in mammals, we have employed microarrays to characterize global gene expression profiles in the mouse brain following acute domoic acid exposure. Approximately 2-2.5% of all the genes expressed in the mouse brain undergo a significant change in expression following domoic acid exposure. Some of the early induced genes include those involved in calcium homeostasis as well as pain and inflammatory responses. Down regulated genes include transcription factors involved in expression of signaling pathways activated by domoic acid. Understanding the coordinated expression and interaction of genes following acute toxicity will provide a better understanding of the mechanisms of neurotoxicity caused by marine algal toxins and mechanisms of neuroprotection. Comparative studies of gene expression profiles in response to different algal toxins are anticipated to yield biomarkers of exposure.

Contact: Fran VanDolah

First Time Identification Domoic Acid in Whale Mortality Event on Georges Bank Expands the Threat of Marine Toxin Exposure to North Atlantic

Between June 17 and July 30, 2003, twelve endangered humpback whales, one pilot whale, and one fin whale were found dead in open waters off Massachusetts near Georges Bank. Tissues and fluids collected from the whales by the NMFS Marine Mammal Stranding Network were tested by the Marine Biotoxins Analytical Response Team for the presence of saxitoxin and domoic acid, two algal toxins known to occur in these waters and previously associated with marine mammal mortality events. Saxitoxin was found at low concentrations in the stomach contents and feces of one humpback whale, while all other samples were negative. The presence of saxitoxin indicates exposure of the whale to this neurotoxin; however, concentrations present were lower than those recorded previously in actively feeding right whales. Saxitoxin is responsible for paralytic shellfish poisoning in humans and is suspected in the 1988 die-off of humpback whales in Cape Cod Bay. Domoic acid was confirmed in the intestinal contents of a different humpback whale at a level similar to those previously documented in the feces of fatally intoxicated marine mammals. Frustules of diatoms from the domoic acid producing genus, *Pseudo-nitzschia*, were also found in the intestinal contents of this animal. Domoic acid is a neurotoxin that causes seizures and permanent brain damage and has been responsible for sea lion, otter, and cetacean mortalities along the California coast. Domoic acid has been identified previously on Georges Bank associated with scallops, leading to closure of that fishery in the mid-1990's. This is the first documentation of domoic acid associated with a marine mammal mortality event in the northeastern U.S. and provides another line of evidence that the range of harmful algal bloom impacts may exceed current estimates. *Contact: Fran VanDolah*

Success of Prototype Remote harmful Algal Bloom (HAB) Sensor Stimulates Development of Second Generation Instrument

The first generation of an autonomous, *in situ* sensor for HAB species and their toxins, called the Environmental Sample Processor (ESP), has been field tested successfully for domoic acid-producing *Pseudo-nitzschia* and for saxitoxin-producing *Alexandrium*. As part of a collaborative project under the multi-agency National Oceanographic Partnership Program (NOPP), scientists from CCEHBR and the Monterey Bay Aquarium Research Institute (MBARI) are now designing a second generation ESP platform that will be smaller and include enhanced sampling/processing capabilities that will increase its flexibility for use in monitoring and research. Membrane-based arrays will provide concurrent, near-real time detection for both organisms and toxins. Data telemetered autonomously from the ESP to land-based facilities will aid in efforts to forecast bloom development and movement, and will ultimately be available through a web-based user interface. *Contact: Greg Doucette*